Detecting Migrants in Populations
of *Rhizoctonia solani* Anastomosis Group 3 from Potato
in North Carolina Using Multilocus Genotype Probabilities

Paulo C. Ceresini, H. David Shew, Rytas J. Vilgalys, Liane Rosewich Gale, and Marc A. Cubeta

First, second, and fifth authors: Department of Plant Pathology, North Carolina State University, Raleigh 27695; third author: Department of Biology, Duke University, Durham, NC 27708; and fourth author: U.S. Department of Agriculture-Agricultural Research Service, Cereal Disease Laboratory, University of Minnesota, St. Paul 55108.
Current address of P. C. Ceresini: Departamento de Biologia, Faculdade de Engenharia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), 15385-000, Ilha Solteira, São Paulo, Brazil.
Accepted for publication 19 December 2002.

ABSTRACT

Ceresini, P. C., Shew, H. D., Vilgalys, R., Gale, L. R., and Cubeta, M. A. 2003. Detecting migrants in populations of *Rhizoctonia solani* anastomosis group 3 from potato in North Carolina using multilocus genotype probabilities. Phytopathology 93:610-615.

The relative contribution of migration of *Rhizoctonia solani* anastomosis group 3 (AG-3) on infested potato seed tubers originating from production areas in Canada, Maine, and Wisconsin (source population) to the genetic diversity and structure of populations of *R. solani* AG-3 in North Carolina (NC) soil (recipient population) was examined. The frequency of alleles detected by multilocus polymerase chain reaction-restriction fragment length polymorphisms, heterozygosity at individual loci, and gametic phase disequilibrium between all pairs of loci were determined for subpopulations of *R. solani* AG-3 from eight sources of potato seed tubers and from five soils in NC. Analysis of molecular

variation revealed little variation between seed source and NC recipient soil populations or between subpopulations within each region. Analysis of population data with a Bayesian-based statistical method previously developed for detecting migration in human populations suggested that six multilocus genotypes from the NC soil population had a statistically significant probability of being migrants from the northern source population. The one-way (unidirectional) migration of genotypes of *R. solani* AG-3 into NC on infested potato seed tubers from Canada, Maine, and Wisconsin provides a plausible explanation for the lack of genetic subdivision (differentiation) between populations of the pathogen in NC soils or between the northern source and the NC recipient soil populations.

Additional keywords: migrant genotypes, population genetics, *Thanatephorus cucumeris*.

Migration and gene flow are important forces that shape evolution of agricultural crop pathogens contributing to the genetic diversity and structure of populations. In the Rhizoctonia pathosystem of potato, mycelium and sclerotia of *Rhizoctonia solani* Kühn anastomosis group 3 (AG-3) associated with potato seed tubers (e.g., seedborne inoculum) provides an efficient mechanism to disperse the pathogen among subpopulations (1). Long-distance dispersal and migration of *R. solani* AG-3 on infested potato seed tubers are postulated to occur from source populations in commercial seed production areas of Canada (Prince Edward Island and New Brunswick) and the northern United States (Maine, Minnesota, New York, North Dakota, and Wisconsin) to recipient soil populations in North Carolina (NC). The migration of *R. solani* AG-3 predominantly from one population into another without an equal amount of migration in the opposite direction is referred to as one-way (unidirectional) migration (13). When migrant individuals of *R. solani* AG-3 from source populations reproduce and become established in recipient populations, the evolutionary process of gene flow has occurred (7,17). Gene flow provides an efficient mechanism to introduce new combinations of genes (alleles) into populations and keeps them from becoming reproductively isolated. Relatively low levels of migration and gene flow are required to prevent genetic divergence among subpopulations

(13). In several plant pathosystems, the lack of population subdivision (differentiation) has provided evidence for pathogen migration throughout a geographic region due to some mechanism of long-distance dispersal of viable propagules (17). To better understand the relative contribution of the annual introduction of genotypes (individuals) of *R. solani* AG-3 on potato seed tubers to the resulting genetic diversity and structure of soil populations in eastern NC, the following hypotheses and questions were formulated. Do migrant genotypes of *R. solani* AG-3 present on potato seed tubers introduced annually into commercial production fields contribute to the overall population of the pathogen present in NC soil? Our hypothesis was that some genotypes of *R. solani* AG-3 present in NC soil are migrants originating from seed potato production areas in Canada, Maine, and Wisconsin. The second question addressed was: Is there evidence for genetic subdivision between source and recipient soil populations of *R. solani* AG-3? Our hypothesis was that migration and gene flow have prevented geographic subdivision between source and recipient populations of *R. solani* AG-3.

MATERIALS AND METHODS

Sampling. Two samples representing seed source and recipient soil populations of *R. solani* AG-3 were analyzed: one from infested seed potato with black sclerotia of the fungus (source population: seedborne) and another from naturally infected potato with symptoms of stem canker (recipient population: soilborne). The recipient soil population was obtained from infected potato plants produced from seed tubers previously disinfested with 2% for-

maldehyde to eliminate seedborne *R. solani* AG-3 (3). The formaldehyde disinfestation treatment was effective in eliminating *R. solani* AG-3 from potato seed tubers planted in fields from which the soil population was sampled (4,5). Infected plant samples were collected from one commercial potato production field in each of five counties in NC (4). To examine the contribution of genotypes present on potato and introduced as seedborne inoculum to the overall population present in NC soils, 100 seed potatoes (originating from seed production farms in Canada [New Brunswick], Maine, and Wisconsin) were randomly collected from storage bins on farms in each of the five NC counties. Three of the five farms had more than one seed lot sampled, totaling eight seed sources (4). These five farms from which the seed tubers were collected were the same five farms whose fields were the source of isolates of *R. solani* AG-3 in the recipient population. Ten seed potatoes naturally infested with sclerotia of *R. solani* were randomly selected from each lot. A total of 58 isolates were obtained from the seed potato sample. Pure cultures of *R. solani* were established and the anastomosis grouping of each isolate was determined using standard protocols (12,14,15).

DNA isolation and determination of genotype. Genomic DNA was extracted according to methods described previously (20). The genotype of each isolate of *R. solani* AG-3 from potato seed tubers was determined with polymorphic codominant single-locus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) markers developed for *R. solani* AG-3 (4). Multi-locus PCR-RFLP genotypes of all isolates were determined using combinations of a specific PCR product and restriction enzymes for each of seven polymorphic loci as described previously (4).

Data analysis. To test for random mating, the source (seedborne) population was analyzed for linkage disequilibrium between all pairs of loci, which was calculated using a test analogous to Fisher's exact test (23,24). Calculations were performed on clone-corrected data using Genetic Data Analysis (GDA) software, version 1.0 (d15) (computer program for the analysis of allelic data provided by P. O. Lewis and D. Zaykin, available on the GDA website).

To identify migrants, or individuals with recent migrant ancestry, a Bayesian-based statistical method that employs multilocus genotypes probabilities was utilized (18). The model determines the relative probability that an individual, m, was born to parents with no recent migration ancestry in population i, rather than population i', which is given by the ratio of the probabilities Λ. Positive values of $\ln\Lambda$ indicate that the null hypothesis (the individual is not a migrant) is favored, whereas negative values indicate that the alternative hypothesis (the individual is a migrant) is favored. The critical (rejection) region for the test of statistical significance was chosen to be $\alpha = 0.05$. The power of the test to reject the null hypothesis when it is false, for specified critical regions α was also estimated. To increase the chances of rejecting the null hypothesis of no migrant ancestry, the minimum acceptable value for power was set to be greater than 0.5 (2,21). Calculations were done using the program *immanc* version 5.14.8.00 (18) (software for testing whether an individual is a migrant or is or recent migrant ancestry provide by B. Rannala and J. Mountain, University of California, Berkeley, available on the *immanc* website).

Between-population analyses were conducted to test for presence of geographic structure in source populations of *R. solani* AG-3 from potato seed tubers compared with the recipient soil population using two independent population genetics measures: (i) analysis of molecular variance (AMOVA) and (ii) pairwise Φ statistics comparisons. AMOVA was used to estimate variance components considering the number of differences between molecular genotypes (8,22,23). These estimates reflect the correlation of diversity at different levels of hierarchical subdivision (e.g., between groups or geographic populations). AMOVA analyses

were conducted using ARLEQUIN version 2.000 (software for population genetics data analysis provided by S. Schneider, D. Roeslli, and L. Excoffier, University of Geneva, Switzerland, available on the ARLEQUIN website). Pairwise Φ statistics comparisons (*F* statistics analogues produced by AMOVA) were used to determine the similarity between individual geographic populations.

RESULTS

Determination of genotype. Genotypes and genotypic counts for each of the eight sources of *R. solani* AG-3 from naturally infested potato seed tubers sampled from storage bins at farms in each of five NC counties before planting are presented in Table 1. Fifty multilocus PCR-RFLP genotypes (MRG) were identified from the potato seed tuber sample ($n = 58$) originating from production areas in Canada (New Brunswick), Maine, and Wisconsin. In contrast, 32 MRG were identified from a sample of infected potato stems ($n = 104$) representing the recipient NC soil population with no evidence of geographic subdivision among any of the five locations sampled (4). In addition, the seven loci analyzed in the sample of *R. solani* AG-3 from naturally infested potato seed tubers (source population) were in linkage equilibrium (data not shown). This fulfills an assumption of random mating between individuals within each population, which is required by the method of Rannala and Mountain (18) for detecting migrants.

The power of posterior probability ratio test for inferring recent migration into the recipient populations of *R. solani* AG-3 from NC is presented in Table 2. A test of the hypothesis that an individual from the NC population is a migrant (generation $d = 0$) had power greater than 0.5 in four population comparisons: Wisconsin 1, 4, and 7, and Maine 5. The posterior probability ratio test was also applied to predict whether specific individuals of *R. solani* AG-3 sampled in NC have Canada, Maine, or Wisconsin ancestry. Six individuals from the 32 MRG identified from NC recipient soil population produced levels of ancestry indicating that they were migrants (Table 3). Three isolates from the NC recipient soil population (P137-Tyrrell, MRG 77; P161-Hyde, MRG 20; and P203-Camden, MRG 72) appeared to be migrants from Maine (sample 5). Two other isolates were indicated to be recent migrants from Wisconsin (P140-Currituck, MRG 80 from sample 1; and P186-Camden, MRG 04 from sample 7). A sixth isolate (P172-Hyde, MRG 42) had significant posterior probability ratio indicating ancestry from Canada (sample 2). However, the power of test to reject the null hypothesis of no migrant ancestry was less than 0.5.

Population structure. Using AMOVA, overall Φ_{ST} between the recipient soil population of *R. solani* AG-3 from NC and from potato seed tubers was 0.002 and not significantly different from zero ($P = 0.39$, data not shown). In general, most of the molecular variation was detected within populations, with minimal variation occurring between the two groups. When the source population was subdivided into sampling units (based on the geographic origin of potato seed tubers), subdivision was only slightly higher among sampling units within than between the two groups (seedborne and soilborne) ($\Phi_{ST} = 0.016$). This value of Φ_{ST} was significantly greater than zero ($P \leq 0.05$).

Pairwise comparison between the source population sample and the NC recipient soil population of *R. solani* AG-3 from potato was performed using estimates of F_{ST} analogues (pairwise Φ_{ST} values) (Table 4). Pairwise Φ_{ST} values for only a few population comparisons were significantly greater than zero, indicating little differentiation or population subdivision. For example, the recipient soil population from NC was significantly different from the source population Wisconsin 1 but very similar to all the other remaining samples. Within the source population, sample Wisconsin 4 was significantly different from the samples Wisconsin 1 and 7, Maine 3, and Canada 2.

DISCUSSION

This study describes the application of a test for detecting migration from source populations (seedborne) of the plant-pathogenic fungus *R. solani* AG-3 from potato into a recipient soil population in NC using MRG. The Bayesian-based statistical method used to analyze migration in this study was originally developed by Rannala and Mountain (18) for detecting immigration of human individuals. This method was postulated as being appropriate for providing relevant information for studying the influence of migration of individuals within populations of animals, humans, and plants for which RFLP markers were used for genotyping. To our knowledge, the method has only been used to examine human populations, not populations of a plant pathogen. However, Fisher et al. (9) have recently modified the method of Rannala and Mountain (18) for use with haploid fungi to identify sources of human infections associated with species of *Coccidioides*. In this paper, we present information on the potential utilization of this method for the study of dispersal of seedborne genotypes of *R. solani* AG-3 from seed potato production areas and the contribution of migrants to the gene pool of a recipient soil population of this pathogen in NC potato fields.

TABLE 1. Observed counts of multilocus polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotypes (MRG) of *Rhizoctonia solani* anastomosis group 3 (AG-3) present on potato seed tubers introduced into North Carolina from eight sources in 1997

MRG[a]	Genotype[b]							Origin[c]								Observed counts
	pP09	pP42	pP45	pP46	pP47	pP83	pP89	Wisconsin 1	Canada 2	Maine 3	Wisconsin 4	Maine 5	Maine 6	Wisconsin 7	Maine 8	
1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	…	…	…	…	1	…	…	…	1
2	1/1	1/1	1/1	1/1	1/1	1/1	1/2	…	…	1	…	…	2	…	1	4
3	1/1	1/1	1/1	1/1	1/1	1/1	2/2	…	…	…	…	…	1	…	…	1
8	1/1	1/1	1/1	1/1	1/1	2/2	1/1	…	…	…	…	…	…	1	…	1
9	1/1	1/1	1/1	1/1	1/1	3/3	1/2	…	…	…	…	1	…	…	…	1
10	1/1	1/1	1/1	1/1	1/2	1/1	1/2	…	…	1	…	…	…	…	…	1
11	1/1	1/1	1/1	1/1	1/2	1/2	1/2	…	…	…	…	…	1	…	…	1
13	1/1	1/1	1/1	1/1	1/2	1/3	1/2	…	…	…	…	…	…	…	1	1
14	1/1	1/1	1/1	1/2	1/1	1/1	1/1	…	1	…	…	…	…	…	…	1
18	1/1	1/1	1/1	1/2	1/1	1/3	1/2	…	…	…	…	…	…	…	1	1
19	?/?	1/1	1/2	1/1	1/1	1/1	1/2	…	…	…	…	1	…	…	…	1
21	1/1	1/1	1/2	1/1	1/1	1/1	2/2	…	1	…	…	…	…	…	…	1
22	1/1	1/1	1/2	1/1	1/2	1/1	1/1	…	…	…	…	…	…	1	…	1
23	1/1	1/2	1/1	1/1	1/2	3/3	1/2	…	…	…	…	…	…	…	1	1
24	1/1	1/1	1/2	1/2	1/1	1/2	1/1	…	…	…	…	…	…	1	…	1
25	1/1	1/1	1/2	1/2	1/1	1/2	1/2	…	…	1	…	…	…	…	…	1
27	1/1	1/2	1/1	1/1	1/1	1/1	1/2	…	…	…	…	…	…	1	…	1
29	1/1	1/2	1/1	1/1	1/2	1/1	1/2	1	…	…	…	…	…	…	…	1
30	1/1	1/2	1/1	1/1	1/2	3/3	1/2	…	…	…	…	…	…	…	1	1
31	1/1	1/2	1/1	1/1	1/2	3/3	1/2	…	…	3	…	…	…	…	…	3
35	1/1	1/2	1/1	1/2	1/1	1/1	2/2	1	…	…	…	…	…	…	…	1
39	1/1	1/2	1/2	1/1	1/2	1/1	1/2	…	…	…	…	1	…	…	…	1
40	1/1	1/2	1/2	1/2	1/1	1/1	1/1	1	…	…	…	…	…	…	…	1
41	1/1	1/2	1/2	1/2	1/2	1/3	1/2	1	…	…	…	…	…	…	…	1
44	1/1	1/3	1/1	1/2	1/1	1/1	1/1	…	1	…	…	…	…	…	…	1
45	1/1	1/3	1/1	1/1	1/1	1/1	2/2	…	…	1	…	…	…	…	…	1
46	1/1	1/3	1/1	1/2	1/2	1/2	2/2	…	…	…	…	…	…	…	1	1
47	1/1	1/3	1/2	1/1	1/1	1/1	1/1	…	…	…	…	…	…	…	1	1
48	1/2	1/1	1/1	1/1	1/1	1/1	1/1	…	1	…	…	…	…	…	…	1
49	1/2	1/1	1/1	1/1	1/1	1/1	1/2	…	…	…	1	…	…	…	…	1
50	1/2	1/1	1/1	1/1	1/1	1/1	1/2	…	…	…	1	…	…	…	…	1
53	1/2	1/1	1/1	1/2	1/1	1/1	2/2	…	…	…	2	…	…	…	1	3
54	1/2	1/1	1/1	1/2	1/1	1/2	1/2	…	…	…	1	…	…	…	…	1
57	1/2	1/1	1/1	1/2	1/2	1/1	1/2	…	…	…	…	1	…	…	…	1
58	1/2	1/1	1/2	1/1	1/1	1/1	1/1	…	…	…	…	…	…	1	…	1
60	1/2	1/1	1/2	1/2	1/1	1/2	1/2	…	…	…	1	…	…	…	…	1
61	1/2	1/2	1/1	1/1	1/1	1/1	1/1	1	…	…	…	…	…	…	…	1
62	1/2	1/2	1/1	1/1	1/1	1/1	1/2	…	1	…	…	…	…	…	…	1
64	1/2	1/2	1/1	1/1	1/1	3/3	1/2	…	…	…	…	…	1	…	…	1
65	1/2	1/2	1/1	1/2	1/2	3/3	1/2	…	…	1	…	…	…	…	…	1
67	1/2	1/3	1/1	1/1	1/1	1/1	1/2	…	…	…	…	…	…	1	…	1
68	1/2	1/3	1/1	1/2	1/1	1/1	1/2	…	…	…	…	…	…	…	1	1
69	1/2	3/3	1/2	1/2	1/1	1/1	1/2	1	…	…	…	…	…	…	…	1
70	2/2	1/1	1/1	1/1	1/1	1/1	1/1	…	…	…	…	…	1	…	…	1
71	2/2	1/1	1/1	1/1	1/1	1/1	1/2	…	…	…	…	1	…	…	1	2
73	2/2	1/1	1/1	1/2	1/1	1/3	1/1	…	…	…	1	…	…	…	…	1
74	2/2	1/1	1/2	1/1	1/1	1/1	1/2	…	…	…	…	…	1	…	…	1
75	2/2	1/1	1/2	1/2	1/1	1/3	1/2	1	…	…	…	…	…	…	…	1
76	2/2	1/1	1/2	1/1	1/1	1/3	1/2	…	…	…	…	1	…	…	…	1
78	2/2	1/2	1/2	1/1	1/1	1/1	2/2	…	…	…	…	1	…	…	…	1
Sample size (*N*)								7	5	8	7	8	7	6	10	58

[a] Numbers from 1 to 78 represent codes for MRG of *R. solani* AG-3 associated with potato seed tubers (source population). Fifty genotypes are included in the table. Another 32 additional MRG were identified from the recipient soil population of *R. solani* AG-3 in North Carolina (4).

[b] Composed by a combination of seven PCR-RFLP-based loci. The codes pP09 to pP89 represent loci. The numbers separated by / are used to define alleles at the seven distinct loci. For example, 1/1 indicates homozygosity for allele 1. Details of the PCR-RFLP genotyping system and allele designation are presented in literature citation 4.

[c] Origin of potato seed tubers infested with *R. solani* AG-3 obtained from five locations in North Carolina prior to planting. Entries correspond to the number of times the indicated genotype was recovered.

According to the model proposed by Rannala and Mountain (18), the NC recipient soil population of *R. solani* AG-3 has likely experienced recent migration. The analysis indicated levels of ancestry for 6 of 32 MRG from the NC recipient soil population that are consistent with the hypothesis that they migrated from a northern source population. However, this conclusion should not be equated with determining the rate of migration because no attempt was made to estimate rates of migration by calculating probabilities from multiple comparisons. This evidence of migration is consistent with the evidence from population structure analyses

indicating that most of the source populations are genetically similar to the NC soil population (4). In addition, of the 10 common genotypes found in both source and recipient populations, seven were among the most frequent genotypes recovered from soil in NC (4).

Given the annual movement of large volumes of potatoes from seed production areas into NC, even relatively inefficient long-distance dispersal would probably produce the level of genetic similarity observed in this study (13). Although the actual efficiency of long-distance dispersal is unknown, our results indicate that migration of *R. solani* AG-3 on potato seed tubers occurs at a rate sufficient to prevent genetic divergence of pathogen populations to ensure that some level of gene flow occurs annually into NC. However, the distinction between recent establishment of the NC population and gene flow is tenuous and should be interpreted with caution.

Gene flow causes the establishment of new alleles or genotypes into new locations through introduction, reproduction, and survival of the introduced organism. Gene flow tends to homogenize alleles among the populations and keeps geographic populations genetically interconnected (19). High rates of migration and gene flow were found among populations of other plant-pathogenic fungi: *Mycosphaerella graminicola* on wheat (17), *Phytophthora infestans* on potato (10,11), and *Magnaporthe grisea* on rice (16). These high levels of gene flow were also probably related to the long-distance dispersal of these pathogens via infected seed, as observed with *R. solani* AG-3. Although these pathogens are associated with seeds or propagation material and can be dispersed great distances, they are also capable of aerial dispersal via

TABLE 2. Power of posterior probability ratio (lnΛ) test for recent migration of *Rhizoctonia solani* anastomosis group 3 multilocus polymerase chain reaction-restriction fragment length polymorphism genotypes from potential source populations into the North Carolina soil population ($\alpha = 0.05$)

Sample population	Potential source	Power at $d = 0$[a]
North Carolina	Wisconsin 1	0.648
	Canada 2	0.334
	Maine 3	0.498
	Wisconsin 4	0.596
	Maine 5	0.707
	Maine 6	0.361
	Wisconsin 7	0.560
	Maine 8	0.496

[a] d is the number of generations in the past that the migration is hypothesized to have taken place. If $d = 0$, the individual under consideration has migrated from a source population. Power indicates the probability of the test to reject the null hypothesis of no migrant ancestry when it is false, for specified critical regions α.

TABLE 3. Power of the posterior probability ratio test to detect migrant ancestry in a population of *Rhizoctonia solani* anastomosis group 3 from potato in North Carolina[a]

			Statistical probabilities of hypothetical individual migrant ancestor[b] ($d = 0$)		
Isolate	Genotype	Potential source	lnΛ	α	Power
P137–Tyrrell	MRG 77	Maine 5	−1.017*	0.046	0.71
P137–Tyrrell	MRG 77	Maine 6	−1.259*	0.046	0.36
P140–Currituck	MRG 80	Wisconsin 1	−2.326*	0.011	0.64
P161–Hyde	MRG 20	Wisconsin 1	−0.605[ns]	0.085	0.62
P161–Hyde	MRG 20	Maine 5	−1.097*	0.043	0.72
P161–Hyde	MRG 20	Wisconsin 7	−1.719*	0.028	0.55
P161–Hyde	MRG 20	Canada 2	−1.790*	0.017	0.37
P161–Hyde	MRG 20	Maine 6	−1.282*	0.046	0.36
P172–Hyde	MRG 42	Canada 2	−1.150*	0.040	0.38
P186–Camden	MRG 04	Wisconsin 7	−1.368*	0.042	0.56
P203–Camden	MRG 72	Maine 5	−1.195*	0.038	0.70
P203–Camden	MRG 72	Maine 6	−1.779*	0.021	0.36

[a] Six individuals with posterior probability ratios lnΛ indicating possible migration (* = significant at $\alpha < 0.05$; [ns] = not significant). MRG = multilocus polymerase chain reaction-restriction fragment length polymorphism genotypes.
[b] d is the number of generations in the past that the migration is hypothesized to have taken place. If $d = 0$, the individual under consideration has migrated from a source population. Power indicates the probability of the test to reject the null hypothesis of no migrant ancestry when it is false, for specified critical regions α.

TABLE 4. Pairwise Φ_{ST}[a] comparison of source populations of *Rhizoctonia solani* anastomosis group 3 (AG-3) from potato seed tubers introduced from eight sources into North Carolina in 1997

Origin[b]	Wisconsin 1	Canada 2	Maine 3	Wisconsin 4	Maine 5	Maine 6	Wisconsin 7	Maine 8
Wisconsin 1	–	…	…	…	…	…	…	…
Canada 2	−0.006	–	…	…	…	…	…	…
Maine 3	0.015	0.044	–	…	…	…	…	…
Wisconsin 4	0.061*	0.064*	0.107*	–	…	…	…	…
Maine 5	0.012	0.020	0.038	0.005	–	…	…	…
Maine 6	0.051	0.011	0.013	0.022	−0.051	–	…	…
Wisconsin 7	0.058	−0.003	0.074	0.126**	0.071	0.050	–	…
Maine 8	0.005	0.006	−0.052	0.047	0.010	−0.003	0.064	–
North Carolina	0.044*	−0.009	0.011	0.044	0.024	−0.012	0.051	−0.003

[a] Population pairwise Φ_{ST} was calculated using ARLEQUIN version 2.000 (University of Geneva, Switzerland). Asterisks indicate significance of *P* values; $P < 0.05$ (*) and $P < 0.01$ (**) leading to values of Φ_{ST} larger than or equal to the observed value when permuting multilocus restriction fragment length polymorphism (RFLP) genotypes (MRG) between populations (1000 permutations were performed). Pairwise comparison with 32 previously identified MRG of *R. solani* AG-3 from the North Carolina soil population (4) was also conducted using clone-corrected data.
[b] Origin of potato seed tubers infested with *R. solani* AG-3 obtained from eight sources from five locations in North Carolina prior to planting.

asexual spores, and in the case of *M. graminicola*, via sexual spores (i.e., ascospores). In contrast, the dispersal of sexual spores (i.e., basidiospores) of *Thanatephorus cucumeris* (anamorph =*R. solani*) is nonexistent or limited. The model of Rannala and Mountain (18) may be potentially useful for detecting migration in other plant pathosystems in which inoculum is associated with seed or plant material. Therefore, a critical examination of populations of pathogens associated with seed or transplants is warranted when conducting population biology and genetic experiments.

An interesting feature of the *R. solani* AG-3 pathosystem on potato is that the migration pattern detected in NC is basically unidirectional, with genotypes originating from the seed potato production areas in the northern United States and Canada. The one-way migration model of gene flow proposed by Hartl and Clark (13) would be appropriate for describing the *R. solani* AG-3 pathosystem on potato in NC. Unidirectional migration from source populations followed by establishment of migrant genotypes in the recipient soil population through colonization, reproduction, and survival would cause levels of gene flow between source and recipient populations to be high. According to this model, only a low level of migration every year would be necessary for a recipient soil population to mimic the structure found in a source population.

Analysis of the data presented in this study support the concept of migration of *R. solani* AG-3 on potato seed tubers into NC and has provided evidence for a low level of genetic differentiation between source and recipient populations. However, the causal role of contemporary gene flow on the observed population structure could not be determined in this study. Therefore, we suggest that the similarity of *R. solani* AG-3 populations is better explained by recent establishment of genotypes from common sites of origin.

An important concern with the application of the method proposed by Rannala and Mountain (18) is that the statistical power of this test is dependent on the number of loci analyzed, the degree of genetic differentiation between populations, and the number of individuals sampled. In the study by Rannala and Mountain (18), 63 loci were examined. However, with greater differentiation between populations, fewer loci are needed to obtain the same power of resolution. In our study, it was inferred that gene flow between recipient NC and source population of *R. solani* AG-3 ($F_{ST} = 0.002$) was occurring which resulted in little differentiation among population samples. Rannala and Mountain (18) stated that their method is appropriate for populations with low genetic differentiation and considered an F_{ST} value of 0.056 as low. This value is 28 times higher than what was observed for the comparison between NC recipient soil and source populations of *R. solani* AG-3 in this study. Because source and recipient soil populations of *R. solani* AG-3 from potato showed almost no subdivision, an increase in the number of loci and individuals analyzed is warranted. The small sample size in this study may have compromised our ability to associate migrants with a specific source population.

Information is lacking on the genetic diversity and population structure of *R. solani* AG-3 from potato on a global (or larger spatial) scale. In the absence of this information, it would be very difficult to identify an external source for the NC populations of *R. solani* AG-3, particularly if little genetic diversity existed on a global scale. However, recent evidence for population subdivision of *R. solani* AG-3 from tobacco and potato on a global basis was found and would provide a foundation for examining source populations (6). DNA sequence variation in two of the markers used on this paper (pP42 and pP89) indicated that while no subdivision was observed between the potato populations from northern United States and eastern NC for the pP42F locus ($\Phi_{ST} = 0.03414$, not significant), differentiation was detected for the locus pP89 ($\Phi_{ST} = 0.25670$, significant at $P < 0.05$) (4). In addition, the same potato populations from northern United States and eastern NC

were significantly different from tobacco populations from central NC or southern Brazil (the overall Φ_{ST} between groups of populations was 0.8811 for pP42F locus and 0.9372 for pP89 [$P \leq 0.001$]).

Incorrect interpretation of experimental data may also result from extensive gene flow between a source population from which the individual originated, but was not included in the sample (18). In our study, we found evidence suggesting that gene flow was occurring between the various source populations of *R. solani* AG-3 (with the exception of sample Wisconsin 4). Gene flow between potential source populations would make it impossible to identify exactly which source populations gave rise to which genotypes in the recipient soil population. However, it is possible that other sources not tested in this study may have also contributed some migrants as a result of gene flow between source populations not sampled.

The method of Rannala and Mountain (18) provided an estimate of the number of migrants in samples from source populations and suggested that 6 of 32 MRG present in the NC recipient soil population were present prior to the arrival of those on infested seed pieces in the same year. This suggests that certain MRG are capable of persisting in soil in the absence of the potato host. Although data analysis suggests that 6 of 32 MRG probably originated from the source population, there is no certainty that all six originated from the same and/or different source populations.

This study provides initial information on the occurrence of gene flow affecting recipient soil populations of *R. solani* AG-3 from potato in NC in comparison with source populations of the pathogen. Studies that incorporate larger sample sizes are needed to elucidate the magnitude of migration and its effects on gene flow in populations of this pathogen. Future research should also include a comparison of gene and genotypic diversity from northern United States (Wisconsin and Maine) and NC populations of *R. solani* AG-3. These areas are responsible for the production of most of the seed potatoes imported into eastern NC and other southeastern U.S. production regions. For inferring population structure of the pathogen at those locations in northern United States and Canada, sampling should also be conducted directly there, instead of sampling introduced seed tubers locally. A more detailed analysis of the population genetics of *R. solani* AG-3 from northern North America could determine the full extent of gene flow and migration from northern to southeastern United States. If higher levels of gene flow are occurring, then populations should exhibit similar levels of gene diversity. However, if gene flow is minimal, then the source population could be genetically more diverse.

LITERATURE CITED

1. Adams, M. J., Hide, G. A., and Lapwood, D. H. 1980. Relationships between disease levels on seed tubers, on crops during growth and in stored potatoes. I. Introduction and black scurf. Potato Res. 23:201-214.
2. Berger, J. O. 1985. Statistical Decision Theory and Bayesian Analysis. 2nd ed. Springer-Verlag, New York.
3. Carling, D. E., Leiner, R. H., and Westphale, P. C. 1989. Symptoms, signs, and yield reduction associated with rhizoctonia disease of potato

induced by tuberborne inoculum of *Rhizoctonia solani* AG-3. Am. Potato J. 66:693-702.

4. Ceresini, P. C., Shew, H. D., Rosewich, L., Vilgalys, R., and Cubeta, M. A. 2002. Genetic structure of populations of *Rhizoctonia solani* AG-3 from potato in eastern North Carolina. Mycologia 94:450-460.

5. Ceresini, P. C., Shew, H. D., Vilgalys, R., and Cubeta, M. A. 2002. Genetic diversity of *Rhizoctonia solani* AG-3 from potato and tobacco in North Carolina. Mycologia 94:437-449.

6. Ceresini, P. C., Shew, H. D., Vilgalys, R., James, T. Y., and Cubeta, M. A. 2001. Lack of gene flow between populations of *Rhizoctonia solani* AG-3 from potato and tobacco suggests they constitute genetically distinct groups within AG-3. (Abstr.) Phytopathology 91(suppl.):S105.

7. Endler, J. A. 1977. On gene flow. Pages 20-29 in: Geographic Variation, Speciation, and Clones. Princeton University Press, Princeton.

8. Excoffier, L., Smouse, P., and Quattro, J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial restriction data. Genetics 131:479-491.

9. Fisher, M. C., Rannala, B., Chaturvedi, V., and Taylor, J. T. 2002. Disease surveillance in recombining pathogens: Multilocus genotypes identify sources of human *Coccidioides* infections. Proc. Natl. Acad. Sci. USA 99:9067-9071.

10. Fry, W. E., Goodwin, S. B., Matuszak, J. M., Spielman, L. J., Milgroom, M. G., and Drenth, A. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. Annu. Rev. Phytopathol. 30:107-129.

11. Goodwin, S. B. 1997. The population genetics of *Phytophthora*. Phytopathology 87:462-473.

12. Gutierrez, W. A., Shew, H. D., and Melton, T. A. 1997. Sources of inoculum and management for *Rhizoctonia solani* damping-off on tobacco transplants under greenhouse conditions. Plant Dis. 81:64-66.

13. Hartl, D. L., and Clark, A. G. 1997. Principles of Population Genetics. 3rd ed. Sinauer, Sunderland, MA.

14. Herr, L. J., and Roberts, D. L. 1980. Characterization of *Rhizoctonia solani* populations obtained from sugarbeet fields with differing soil textures. Phytopathology 70:476-480.

15. Kulik, M. M., and Dery, P. D. 1995. Use of DAPI for anastomosis grouping typing of strains of the fungus *Rhizoctonia solani*. Biotech. Histochem. 70:95-98.

16. Levy, M., Romao, J., Marcheti, M. A., and Hamer, J. E. 1991. DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95-102.

17. McDermott, J. M., and McDonald, B. A. 1993. Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31:353-373.

18. Rannala, B., and Mountain, J. L. 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. USA 94:9197-9201.

19. Rogers, S. O., and Rogers, M. A. M. 1999. Gene flow in fungi. Pages 97-121 in: Structure and Dynamics of Fungal Populations. Kluwer Academic Publishers, Dordrecht, the Netherlands.

20. Rosewich, U. L., Pettway, R. E., McDonald, B. A., and Kistler, H. C. 1999. High levels of gene flow and heterozygote excess characterize *Rhizoctonia solani* AG-1 IA (*Thanatephorus cucumeris*) from Texas. Fungal Genet. Biol. 28:148-159.

21. Steel, R. G. D., Torrie, J. H., and Dickey, D. A. 1997. Principles and Procedures of Statistics: A Biometric Approach. 3rd ed. McGraw-Hill, New York.

22. Weir, B. S., and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

23. Weir, B. S. 1996. Genetic Data Analysis. 2nd ed. Sinauer, Sunderland, MA.

24. Zaykin, D., Zhivotovsky, L., and Weir, B. S. 1995. Exact tests for association between alleles at arbitrary numbers of loci. Genetica 96:169-178.